Potential biological markers of gestational trophoblastic diseases

Ph.D. theses

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SUMMARY

Gestational trophoblastic diseases are a spectrum of pregnancy related, invasive and noninvasive proliferative trophoblastic disorders, which include partial mole, complete mole and gestational choriocarcinoma. Despite important advances in the diagnosis and therapy of these diseases, molecular pathogenesis has remained largely unknown.

Our purpose was to identify potential differences in gene expression between normal placenta and gestational trophoblastic tumors. „Atlas human cDNA expression array” membrane hybridization technique was used to study the gene expression pattern in normal trophoblast and choriocarcinoma cell lines. As compared to normal trophoblast cells, 6 genes were upregulated and 3 genes were downregulated in choriocarcinoma cells. The downregulation of Hsp-27 in choriocarcinoma cells was confirmed both in vitro with cell lines and in vivo with paraffin sections using RT-PCR, Western-blot and immunohistochemical methods. Furthermore the expression of c-erbB proteins, and matrix metalloproteinases and their inhibitors were invastigated with paraffin sections by immunohistochemistry.

Our findings summerized in this thesis are the following:
1. Atlas cDNA array is a useful and suitable technique to identify differentially expressed genes in normal and malignant cells or tissues, and suitable for search after new biological markers as well.
2. Hsp-27 may play an important role in the genesis and the development of early placenta and the differentiation of trophoblasts. Furthermore, the downregulation of Hsp-27 in choriocarcinoma may contribute to the extreme sensitivity of malignant trophoblastic tumors to chemotherapy.
3. Supposingly c-erbB protein family may be important in the pathogenesis of gestational trophoblastic diseases and in the progression to persistent postmolar tumors.
4. The expression of matrix metalloproteinases and their inhibitors may be related to the development of gestational trophoblastic diseases.
INTRODUCTION

Gestational trophoblastic diseases are a spectrum of pregnancy related, invasive and noninvasive proliferative trophoblastic disorders, which include partial mole, complete mole and gestational choriocarcinoma. These tumors have varying propensity for local invasion and distant spread and despite widespread metastases are highly curable with chemotherapy. Despite important advances in the diagnosis and therapy of these diseases, molecular pathogenesis has remained largely unknown. While the risk of developing choriocarcinoma is increased after complete mole, there is no any marker predict the progression of hydatidiform moles.

Cell proliferation is a complicated process which is coordinated by the interaction of determined protooncogenes and tumor suppressor genes. The limits are blurred between the physiological oncogene expression in early placenta and the changes of oncogene expression in gestational trophoblastic diseases, and the explication of these differences remain unknown. Both activation of some protooncogenes and inactivation of some tumor suppressor genes were already demonstrated in gestational trophoblastic tumors, too. In several tumor systems, protoonogene’s, or tumor suppressor gene’s mutations, can be implicated in the events of neoplastic transformation and tumor progression. The identification of differentially expressed protooncogenes, tumor suppressor genes, and other functional genes and their products in normal placenta and gestational trophoblastic tumors may provide important insight into the pathogenesis of this disease and is worthy of further investigation.
OBJECTIVES

My purpose was to identify potential differences in important functional genes and their products’ expression, behavior and possible pathogenetical role in normal placenta and gestational trophoblastic diseases from the following aspects.

1. „Atlas human cDNA expression array” membrane hybridization technique was used to compare gene expression pattern in normal trophoblast cells with choriocarcinoma cell line, to find potential differences from 588 known functional genes.

2. Heat shock protein – 27 (Hsp-27) expression was studied in normal placenta and choriocarcinoma in vitro and in vivo, because Hsp-27 has a known role in the development of chemoterapeutic drug resistance in human malignancy.

3. The expression of c-erbB proteins was investigated in normal placenta and gestational trophoblastic diseases. Furthermore, these proteins were studied to determine their potential role in the development of postmolar gestational trophoblastic tumor and clinical outcome.

4. The expression of matrix metalloproteinases (MMP) and their inhibitors was investigated in normal placenta and gestational trophoblastic diseases. We also evaluated whether the differences in expression of these enzymes were associated with the development of postmolar gestational trophoblastic tumor and the prognosis of the disease.
EXPERIMENTAL METHODS

1. Human normal trophoblast (3A-Sub-E) and choriocarcinoma (Jar, JEG-3) cell lines growing.

2. Clinical data and tissue samples: This study includes a total of 51 patients with gestational trophoblastic disease and 11 patients with therapeutic first trimester abortion. Of the patients with gestational trophoblastic disease, 16 had partial mole, 25 had complete mole and 10 had choriocarcinoma. Eight cases (32.0%) of the patients with complete mole developed persistent postmolar gestational trophoblastic tumor.

3. Total RNA and messenger RNA isolation from normal trophoblast and choriocarcinoma cells.

4. Nuclear and cytoplasmic protein extraction from the cell lines.

5. “Atlas human cDNA expression array” membrane hybridization technique was used to study the gene expression pattern in normal trophoblast and choriocarcinoma cell lines.

6. The expression of Hsp-27, 60S Ribosomal protein L6 (RP-L6) and DNA repair protein (HIP-116) in cell lines were studied by reverse transcriptase polymerase chain reaction (RT-PCR).

7. To confirm Hsp-27 expression data in cell lines by Western blot analysis.

8. Paraffin sections were studied immunohistochemically for expression of Hsp-27, EGFR, c-erbB-2, c-erbB-3, c-erbB-4, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, and TIMP-1 (Tissue inhibitor of metalloproteinase-1). The expression of Hsp-27 in cell lines was also determined by immunohistochemical analysis.

9. The presence of EGFR in paraffin sections was studied using in situ mRNA hybridization.
RESULTS

Our finding summerized in these results are the following:

1. „Atlas human cDNA expression array” membrane hybridization technique was used to identify differences in the expression pattern of 588 known genes between normal trophoblast and choriocarcinoma cell lines. Nine strong differentially expressed genes were found. Three genes were upregulated in normal trophoblast cells: Hsp-86, Hsp-27, and Fibronectin receptor β-subunit (FR-β). Six genes were upregulated in choriocarcinoma cells: Protooncogene c-myc, NF-κB transcription factor p65, GATA-2 transcription factor, RP-L6, HIP-116, and human nerve growth factor (hNGF). Furthermore, in three randomly selected cases from these genes the observed hybridization results were confirmed using RT-PCR analysis.

2. Hsp-27 was observed to be upregulated in normal trophoblast cells by Atlas array technique and this observation was confirmed in nucleid acid and protein levels in vitro in cell lines and in vivo with paraffin sections using RT-PCR, Western blot, and immunohistochemical analyses. The results were similar, strong expression of Hsp-27 in placenta and the downregulation of Hsp-27 in choriocarcinoma were found.

3. The levels of expression of EGFR in choriocarcinoma and syncytiotrophoblasts and cytotrophoblasts in complete molar pregnancy were significantly greater than syncytiotrophoblasts and cytotrophoblasts in both partial mole and normal placenta. In situ hybridization for EGFR mRNA correlated with immunostaining for EGFR in all tissues studied. The immunoreactivity of c-erbB-2 were also significantly stronger in choriocarcinoma and extravillous trophoblasts in complete mole than extravillous trophoblasts in partial mole and normal placenta. The tissues of placenta and gestational trophoblastic tumors demonstrated similar immunostaining for c-erbB-3 and c-erbB-4. Finally, strong expression for EGFR and c-erbB-3 in extravillous trophoblasts of complete mole were found to be significantly correlated with the development of persistent postmolar disease.
4. Choriocarcinoma exhibited significantly stronger expression for both MMP-1 and MMP-2 than syncytiotrophoblast in normal placenta, partial mole and complete mole and extravillous trophoblast in placenta. Furthermore the extravillous trophoblast in partial and complete mole had significantly stronger staining for MMP-2 than the extravillous trophoblast in normal placenta. The expression of MMP-3, MMP-9, and MMP-13 were similar in all four tissues. TIMP-1 expressed significantly stronger in normal placenta, partial and complete mole than in choriocarcinoma. Finally no correlation was found between the immunoreactivity of these enzymes and the development of persistent trophoblastic diseases.
CONCLUSIONS

Our results summarized in the following conclusions:

1. Our results showed that Atlas cDNA array is a useful and suitable technique to identify differentially expressed genes in normal and malignant cells. Furthermore it is suitable for search after new biological markers as well, which could be important diagnostic and prognostic factors in predisposition of malignant diseases. Our data referred that the identification of differentially expressed genes may provide important insight into the pathogenesis of gestational trophoblastic diseases and worthy of further investigation.

2. Hsp-27 is expressed in high concentration in early placenta and our data supported this observation. Our results confirm that Hsp-27 may play a role in the genesis and the development of early placenta, and in the differentiation of trophoblasts.

   Otherwise Hsp-27 may also be important in the acquisition of chemotherapeutic drug resistance. The downregulation of Hsp-27 in choriocarcinoma explain the extreme sensitivity of trophoblastic tumors to chemotherapy. Further functional studies should be necessary for verification of this suppose. If it’s true, supposingly at the time of the diagnosis of the disease can recognise the chemotherapy resistance cases and more effective combinations of chemotherapeutic drugs or new and novel therapeutic possibilities can be used for these patients.

3. In conclusion, the expression of EGFR related c-erbB oncogen family play an important role in the pathogenesis and appearance of gestational trophoblastic diseases. Furthermore the increased expression of EGFR and c-erbB-3 proteins in complete mole, as an early marker may also influence the development of persistent gestational trophoblastic tumors after evacuation.

4. Our data showed that MMPs and their tissue inhibitors have an important role in the pathogenesis and progression of gestational trophoblastic diseases. Finally it seems no correlation between MMPs and the development of persistent trophoblastic diseases after evacuation.
1. Foreign papers connected with the dissertation:


2. Hungarian papers connected with the dissertation:


3. Other papers:


4. Cited abstracts:


